1971

Cytology of the Adenohypophysis of the Blue Spiny Lizard, Sceloporus cyanogenys

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CYTOLOGY OF THE ADENOHYPOPHYSIS OF THE BLUE SPINY LIZARD, *SCELOPORUS CYANOGENYS*

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

Marie Potts

1971

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

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ACKNOWLEDGMENTS

The writer wishes to express her appreciation to Dr. Ian P. Callard, under whose guidance this investigation was conducted, for for his patient guidance and criticism throughout the investigation. The author is also indebted to Dr. Robert E. L. Black and Dr. Joseph L. Scott for their careful reading and criticism of the manuscript.

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ABSTRACT

The cytology of the pars distalis of the female iguanid lizard, Sceloporus cyanogenys was studied using a variety of staining techniques. In addition to the chromophobes, four chromophils were distinguished (acidophils type I and II and basophils type I and II). The distribution of the various cell types in the adenohypophysis and alterations in distribution of the cell types correlated with various phases of the reproductive cycle were described. Cytological changes occuring in the adenohypophysis following castration of both preovulatory and post-ovulatory animals were noted. Typical castration changes occurred in the basophil type I, although the reaction to castration was more marked when the operation was performed prior to ovulation. Injection of estrogen ameliorated the post-castration changes, but vacuoles were not completely eliminated from the cells. Type II basophils were only slightly influenced, if at all, by castration. Alterations in the acidophil class were also observed following castration. In the preovulatory animal, there appeared to be a reduction in the erythrosin positive cell numbers following castration with little alteration of the orangeophilic cell type. In contrast, following castration of the pregnant animal, the orange G cell type was markedly reduced in numbers and the erythrosin positive cell unchanged. Injections of estrogen in the castrate preovulatory

animal appeared to increase the number of orange G positive acidophils. The four cell types were tentatively identified as a somatotroph (acidophil-I), a lactotrope (acidophil-II), a gonadotrope (basophil-I) and a thyrotrope (basophil-II).

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INTRODUCTION

The early work on the reptilian adenohypophysis and its cell types has been reviewed by Poris and Charripper (1938). In Anolis carolinensis (Poris and Charripper, 1938), three cell types were recognized. These were identified as acidophils, basophils and chromophobes. Altland (1939) in Sceloporus undulatus undulatus noted that acidophils were the most numerous cell type; further they could be subdivided into red and orange tinctorial classes by the Servinghaus method which employs aniline acid fuchsin. In the snake, Thamnophis sirtalis sirtalis, Hartmann (1944) recognized four distinct cell types, and described two distinctly different acidophils. Cycles of activity were observed in both acidophilic and basophilic cell types. Changes in the latter appeared to be associated with sexual activity. Subsequently, Cieslak (1945) in Thamnophis radix and Miller (1948) in Xantusia vigilis made similar observations to those of Hartmann (1944). However, in Anolis and Thamnophis, carmine positive cells were found in the cephalic region, whereas in Xantusia, this type was found in the caudal region.

More recently, studies by Grignon (1963) and St. Girons (1963) have extended these early observations on the reptilian pituitary. Further, Licht and Nicoll (1969) and Licht and Rosenberg (1969) have used a combination of classical techniques and hormone bioassay methods

in an attempt to refine the ideas of the identity of the different cell types of the reptilian adenohypophysis. Dyeson (1970) has added a tinctorial description and tentative identification of five cell types in Agama agama.

Recent studies (Callard, Bayne and McConnell, 1971; Doolittle and Callard, 1971) have indicated a role for both estrogen and progesterone in the feedback control of the gonad via the pituitary gland in female Sceloporus cyanogenys. The present investigation is an attempt to identify the various cell types in the pituitary of this species as an aid in further understanding the role of various hormones and their action in the control of reptilian reproductive processes.

MATERIALS AND METHODS

Adult female Sceloporus cyanogenys, the ovoviviparous blue spiny lizard, were obtained from a commercial supplier in Texas during December. The animals were housed in 20 sq. ft. enclosures on a bedding of "Sanicel" (Paxton Processing Co.). Room temperature was maintained at 28±2°C during the day, falling to 22±2°C during the night. A 250 watt heat lamp was suspended at the edge of the pen and allowed a maximum of 37°C at the floor with a decreasing gradient across the pen. Shade was supplied with water available ad libitum. Heat lamps and overhead fluorescent lights were automatically controlled on a 12 hour light-12 hour dark regime. Animals were fed commercially supplied crickets daily. Animals were grouped for experimentation in 60 x 60 x 30 cm wire mesh cages, with other conditions as described above.

Castration and hormone injection

Subcutaneous injections of estradiol 17 beta were made in 0.1 ml sesame oil, 10 µg/day, for 14 days.

For castration, animals were anesthetized with "Nembutal" (2.5 mg/100gm/body weight) and a minimum of 30 minutes at refrigerator temperature. Using 70% ethanol asepsis small bilateral incisions were made in the lower ventro-lateral body wall and the ovary pulled to the surface of the body. A ligature was placed between gonad and

adrenal and the gonad removed.

Experimental groups

Pituitaries from a total of thirty-three animals were serially sectioned in situ. Six classes of animals were used.

1. Preovulatory animals (n=6). Autopsy on arrival in the laboratory showed well-developed follicles in the ovaries of these animals.

2. Pregnant animals (n=5). All pregnant animals had well-developed motile embryos in the oviducts. Three animals had been maintained in the laboratory from December 22 until March 28 when they were sacrificed. Two were sacrificed during April within a week of arrival in the laboratory.

3. Post-partum animals (n=6). These animals had been maintained in the laboratory from December 22 and gave birth to living litters (mean litter size 15) prior to March 21.

4. Preovulatory animals castrated on day 0 (December 22) (n=5). The animals were maintained in the laboratory until March 21. All animals had oviducts significantly smaller than initial preovulatory controls at autopsy.

5. Preovulatory animals castrated on day 0 and injected with estradiol 17 beta (December 22) (n=6). Forty-five days post-castration, animals were daily injected with 10 µg estradiol 17 beta subcutaneously for 14 days and were killed on day 60.

6. Pregnant animals, castrated on December 22 (n=6). These animals were autopsied on March 15. Four animals had viable embryos near full-term, and two others had fully developed but dead embryos in the oviducts.

Histology

Animals were sacrificed by decapitation. The heads were then placed immediately into Bouin-Holland sublimated fixative. After fixation for seven days, the skin and outer layers of bone and tissue were removed care being taken not to disturb the position of the brain. The trimmed heads were placed in a 5% formic acid, 5% formaldehyde solution which was changed daily for fourteen days. The heads were embedded in parafin and serially sectioned longitudinally at 6 μ so that the pituitary might be studied *in situ*. Four sections were mounted on a slide with contigous slides being stained with Cleveland-Wolfe trechrome, PAS-orange G-Gurr's Alcian blue at pH 0.2 and 3.0, PAS-orange G, and Gabe's AF with Halmi's counterstain.

Rationale for the use of different stains in the identification of different cell types in the adenohypophysis

The two major classes of cells in the vertebrate adenohypophysis are basophils and acidophils. The first type contains a secretory product which is typically glycoprotein in nature and includes those cells secreting follicle stimulating hormone (FSH), luteinizing hormone or interstitial cell stimulating hormone (LH or ICSH), and thyroid stimulating hormone (TSH). The second class contains two cell types, those secreting growth hormone or somatotropic hormone (GH, STH) and those secreting prolactin (lactotropes, prolactin cells). A differentiation may be made between these two major classes of cells using the periodic acid Schiff reagent (PAS, McManus, 1945) which stains the glycoproteins of the basophilic cell type an intense red to magenta color. The secretory products of the acidophilic cells are simple proteins and these materials typically stain with orange G, azocarmine,

acid fuchsin or erythrosin. Thus a combination of different stains will allow a differentiation of the different classes of cells. However, since both major classes are composites, additional methods must be used to separate the members of each class. Thus, in this investigation the following staining techniques were used.

a. Cleveland-Wolfe trichrome method. This contains orange G, erythrosin and aniline blue. The acidophilic class of cells take up orange G or erythrosin, and the basophils stain more or less similarly with aniline blue. Of the acidophils, as a general rule the growth hormone cell is orange G positive and the prolactin cell erythrosin positive. Thus this combination of stains allows some degree of resolution of the acidophils and the basophils.

b. PAS-alcian blue (Gurr)-orange G. As indicated above, PAS typically stains glycoprotein material present in basophils. The combination of PAS and alcian blue was first introduced by Mowry (1956) who showed that this technique colored acid mucoproteins blue and neutral mucoproteins red. The technique was successfully applied to tetrapod pituitaries by Herlant (1960) and has been useful in some species for distinguishing between FSH and LH cells. Typically, LH cells do not stain with alcian blue, but are PAS positive, whereas the FSH cells have a very mild affinity for the alcian blue at pH 0.2 and a strong affinity for alcian blue at pH 3.0. Thyrotrophs have been shown to have a strong affinity for alcian blue at both pH's. Thus using this staining technique, combined with orange G and a hematoxylin nuclear stain, one can possibly differentiate the basophils further at the same time that some of the acidophils take up orange G.

c. Gabe's aldehyde fuchsin (AF) with Halmi's counterstain. Using

aldehyde fuchsin, it has been possible to stain the thyrotropic cells of the rat and leave the other basophils (the gonadotropic cells) unstained (Gomori, 1950; Halmi, 1951). AF stains cytoplasmic granules which are also alcian blue and aniline blue positive.

d. PAS orange G. Using this combination, in the absence of alcian blue, it is possible to determine whether alcian blue positive material is also PAS positive. In the presence of alcian blue, the reaction to PAS is masked.

The application of these and other cytological techniques to the vertebrate adenohypophysis has been used with great success to differentiate the cells responsible for the production of pituitary tropic hormones. The use of these stains in the present investigation is based on a certain degree of success of these techniques when used in other lower vertebrates as well as mammals.

RESULTS

General topography

The pituitary gland of *S. cyanogenys* lies in a distinct sella turcica formed by the basisphenoid bone. Most anteriorly the gland is overlaid by the relatively fragile presphenoid cartilage of the basisphenoid bone. The adenohypophysis has a relatively small rostral region which extends anteriorly toward the caudal portion of the median eminence from which the portal vessels pass in a bundle into the anterior lobe. Posteriorly the gland enlarges and becomes somewhat keel-shaped. The intermediate lobe is prominent and attached to the anterior lobe caudally and reflected rostrally. The sac-like neural lobe overlies the rostral adenohypophysis and meets the intermediate lobe caudally (Fig. 1).

Cell types

This study revealed the presence of four distinct chromophilic cell types in addition to the chromophobes: two acidophils (A-I and A-II) and two basophils (B-I and B-II) (Table 1).

The type I acidophil is orange G positive (Fig. 7), and two subtypes may be recognized in many specimens (Fig. 6) (a) large, columnar cell arranged in groups or arcades around or along capillaries. This cell is well granulated, the granules staining brilliantly with orange G in the Cleveland-Wolfe trichrome method. This cell is typically confined to the rostral region of the gland, intergrading somewhat

abruptly in the mid-region of the gland with the smaller cell. The second subtype (b) is found throughout the remainder of the gland with a similar type of distribution to the first, but tending to be found either in small groups or singly. This cell is not as markedly columnar and is considerably smaller than the first, staining a dull brick red with orange G.

The type II acidophil is erythrosin positive and PAS positive (Figs. 5 and 8). Its distribution was always rostral, extending down into the ventro-medial region. The cells are columnar in appearance and are arranged similarly to the type Ia acidophil in the vicinity of the capillaries. Those cells found outside of the rostral region are smaller and tend to be found either singly or in small groups.

The type I basophil is distributed throughout the gland (Fig. 2). It is a large ovoid cell, present in clusters and stains with PAS, alcian blue and aniline blue (Fig. 7). Its affinity for alcian blue appears to be dependent upon the functional state of the cell as described below. The second basophil (type II) is typically confined to a band across the mid-region of the gland, sometimes extending more caudally, particularly in the ventral region of the gland (Figs. 3 and 4). This cell was found to be aniline blue, alcian blue, PAS and aldehyde fuchsin positive, and usually either rounded or tear drop in shape, although occasionally columnar. The granulation in this cell type is coarse, often flocculant and stains intensely with PAS in the absence of alcian blue (Fig. 8).

Preovulatory phase

Both types of acidophils were prominent, with abundant and

relatively coarse granulation, and were distributed as described above (Fig. 1). Basophils were extremely prominent and were seen as large rounded cells distributed throughout the gland (Figs. 2 and 9). However, only one type could be distinguished with any certainty. With alcian blue at pH 0.2 only a very few weakly alcian blue positive cells were noted in the mid-region of the gland and all the other basophils were PAS positive. However, with alcian blue at pH 3.0, all of the basophils stained strongly with alcian blue and corresponded to those staining with aniline blue.

Pregnancy

No alterations in the two acidophil types were observed in the pregnant animal. However, following PAS-alcian blue staining it was possible to distinguish two basophils without difficulty. Type I was negative for alcian blue at both pH's tested and was PAS positive. These cells were well granulated and found with the same distribution as the basophils described above for the preovulatory animal. The basophil type II was weakly alcian blue positive at the high pH and strongly alcian blue positive at the low pH. Granulation was coarse and flocculant in many cells. This cell type was found predominantly in the mid-region of the gland intermixed with type I. In the absence of alcian blue this cell type was PAS positive and also stained with aldehyde fuchsin.

Post-partum animals

The acidophil population was not noticeably different from that noted in the other classes of animals with regards to either distribution, numbers or granulation. The basophil type I was present in large numbers extending rostrally amoung the type II acidophils

(Figs. 3 and 4) but as in the pregnant animal was PAS positive and alcian blue negative. Granulation was prominent and affinity for PAS strong. The type I basophils found in the caudal region of the gland appeared to be smaller and to have somewhat less affinity for PAS. Alcian blue positive cells were again restricted to the mid-region of the gland predominantly, and granulation in the post-partum animals appeared stronger than in the pregnant animals.

The effects of castration

A. Before ovulation. Compared to the three reproductive phases described above, alterations in the acidophil type II population were noted. Erythrosinophilic cells appeared very much reduced in numbers, and in some specimens were almost totally absent. The type I acidophil appeared marginally affected. Basophils of type II were only slightly influenced by castration, if at all. At both pH 0.2 and 3.0 granulation was observed, but appeared to be less than in the intact animals. The number of cells which reacted positively to alcian blue was similar to intact animals as was the distribution of these cells in the gland. In contrast, type I basophils were markedly affected. PAS positive material was extremely sparse and many cells were vacuolated with typical signet ring cells being observed in the mid-ventral and caudal regions of the gland (Figs. 10 and 11). In one animal of this group, a situation similar to that described for the intact preovulatory animal was seen as regards the basophil affinity for alcian blue. That is, large numbers of PAS positive basophils were seen following PAS-alcian blue at pH 0.2, but at pH 3.0 all of the basophils were alcian blue positive. These cells were often vacuolated, but no signet ring cells were observed. The distribution of aldehyde fuchsin

positive cells was similar to that described before.

B. During pregnancy

In four out of six animals, a marked diminution in the number of orange G positive cells was noted, the subtype a being most affected. In at least one instance the gland was almost devoid of orange G positive material. In contrast, type II acidophils showed a typical distribution pattern and were well granulated. The type II basophil was not influenced by castration during pregnancy, and the type I basophil was influenced by the operation to a lesser extent than that described above for the preovulatory animal. Moderate amounts of PAS positive material were present, but the degree of depletion and vacuolation was much less than in the preovulatory castrates. A few signet ring cells were observed.

The effects of castration followed by estrogen injection in the preovulatory animal

Compared to other groups of castrates, orange G positive type I acidophils were much increased in numbers. Further, the caudal type, usually much smaller than the rostral type, was enlarged so that the distinction between these two types was unclear in three of the animals. The erythrosin positive cells appeared to be restored to normal following estrogen injection. Basophils type I were well granulated, almost devoid of vacuoles, and signet ring cells were not observed (Fig. 12). There was a paucity of alcian blue positive granulation in the type II basophils and vacuoles were apparent.

DISCUSSION

Acidophils

In Sceloporus, orange G positive cells were distributed throughout the gland, those in the rostral region being larger and more brilliant in their response to orange G. This difference may be due to the proximity of the rostral cells to the point of entry of the hypophysial portal vessels and may also be a reflection of a quantitative difference in the amount of hypophysiotropic factor reaching the cell from the hypothalamus. Similar observations have been made by Grignon and van Oordt (1963). A correlate of this may be the large numbers of chromophobes present in the caudal region of the gland. These cells probably correspond to the alpha cells of St. Girons (1963) which were described as being generally located in the caudal region of the reptilian hypophysis and reacted with acid dyes. Recently, Licht and Nicoll (1969) demonstrated the presence of a presumptive STH band following polyacrylamide gel electrophoresis of extracts of the caudal region of the adenohypophysis of the turtle Pseudemys scripta elegans. Subsequently, the presence of STH activity in the caudal region of Anolis pituitary was demonstrated by bioassay methods in hypophysectomised Anolis by Licht and Rosenberg (1969), suggesting very strongly that the caudal acidophil is the somatotrope. In contrast is the study of Eyeson (1970) who has suggested that in Agama agama the rostral acidophil is the somatotrope.

In castrated lizards, caudal orange G cells appeared to be smaller and diminished in numbers compared to intact animals, but in castrates injected with estrogen, there was an increase in the number of orange G cells. Most of them were of the larger variety which extended into the caudal region. These changes in the orange G cells are suggestive of a sensitivity of the cells to estrogen. Recently, Zambrano and Deis (1970) have shown that both prolactin and growth hormone cells in the rat hypophysis increase their secretory activity in response to estrogen implants in the median eminence and arcuate nuclei. Since recent studies in this laboratory have indicated a role for growth hormone in association with estrogen in vitellogenesis and ovarian growth in lizards (Callard and Zeigler, 1970; Callard and Banks, 1970), the possibility that estrogen acts in some way to influence the secretory activity of growth hormone secreting cells bears consideration. The distribution of the acidophil type II in Sceloporus correlates well with that described for a similar cell in several reptiles by Licht and Nicoll (1969). These authors further correlated the presence of this cell with the presence of a prolactin band using polyacrylamide gel electrophoresis and prolactin activity using the pigeon crop sac test. Eyeson (1970) condiders the rostral acidophil of Agama to be the somatotrope. In the present study, the erythrosin positive cell was the most stable of the cell types, an alteration in number was noted only following castration in the preovulatory animal. This might suggest some role of the gonad in the control of this cell type, an idea thoroughly authenticated in mammalian studies (Sar and Meites, 1967).

Although the study of Eyeson (1970) tentatively identified the

rostral acidophil as the somatotrope and the caudal acidophil as the lactotrope, the evidence from other workers described above suggests the reverse of this may be true. The strongest evidence against the interpretation made by Eyeson is the bioassay data of Licht and Nicoll (1969). Further, the reactions described by Eyeson for the two cell types (rostral type, erythrosinophilic; caudal type, orangeophilic) is the same as that described here. Eyeson's rationale for assigning the lactotropic identity to the caudal cell is based on alterations in this cell type during the reproductive cycle. However, as suggested above, there is reason to believe that the somatotrope may have an important role in vitellogenesis in lizards and thus alterations in the activity of this cell type may occur during the reproductive cycle.

Basophils

The activity of the basophil-I during the ovarian growth and vitellogenic phase suggests a possible gonadotropic (FSH?) activity for this cell type. In male Anolis carolinensis Licht and Rosenberg (1969) identified gonadotropic activity throughout the adenohypophysis using bioassay techniques. However, the study did not allow for the separation of LH and FSH secreting cell types. Subsequently, Licht (1970) demonstrated that FSH alone can cause follicular growth and ovulation in hypophysectomised Anolis which has led him to suggest that there is only a single FSH-like gonadotropin or gonadotropin complex in lizards. This is in contrast to the suggestion of St. Girons (1963) that the presumptive LH cells are the rostral gamma or X cell and that the presumptive FSH or beta cells can be identified. In this study we were only able to differentiate two types of basophils,

and cytological changes associated with the reproductive cycle suggested only one basophilic type (B-I) associated with reproduction and ovarian growth. The reaction of this cell type to castration with typical vacuolation and signet ring formation suggests a gonadotropic role for this cell but does not aid in the elucidation of a second gonadotrope.

The alteration in the reaction of the basophil-I to alcian blue depending upon the functional state of the gland may be explained if one considers the alcian blue material to be the active secretory product in an otherwise carbohydrate or glycoprotein (which is therefore PAS positive) rich cell. Enzymatic digestion with diastase prior to PAS treatment would determine whether the PAS positive material were a simple carbohydrate. Thus, in the preovulatory animal, large amounts of alcian blue material are present during the phase of ovarian growth. Following ovulation, during pregnancy and in the post-partum animal, the active component (the alcian blue material) may have been discharged. Under these conditions the cells are PAS positive and alcian blue negative. The course of the reaction to castration in the basophil type I, a presumptive gonadotrope, is indicated in the one animal in which even though castrated, the cells contained alcian blue positive material and were at the same time vacuolated. Thus, in this animal, the castration changes had not reached the stage of complete exhaustion, the cells still being able to synthesize their secretory product.

Although St. Girons (1963) considers the rostral basophil (gamma cell) to be the LH cell, the bioassay studies of Licht and Rosenberg (1969) suggest that ICSH (LH) activity is more abundant in the caudal

region of Anolis pituitary and that ACTH is the only pituitary hormone other than prolactin with a rostral distribution in Anolis. Licht and Bradshaw (1969) have suggested that the rostral gamma cell is the corticotroph. The present study does not contribute significantly to the knowledge of the presumptive ACTH cell in lizards in so far as it was not possible to separate a rostral basophil from a caudal basophil. The second basophilic cell type identified in this study is probably the thyrotrope.

In other reptiles, this cell has a similar distribution. In as much as the basophil-II did not show obvious fluctuations which could be correlated with the reproductive cycle or marked alterations following castration and estrogen treatment this cell is unlikely to be associated with direct control of the gonad. St. Girons (1961) located the presumptive FSH cell in the medio-ventral and lateral areas of the pars distalis and this distribution is very close to that described for the basophil-II in this study. On the basis of their histological and bioassay studies, Licht and Rosenberg (1969) suggest that the presumed FSH cell of St. Girons is in fact the TSH cell, which has a predominantly caudal distribution in Anolis. This is corroborated by the studies of Forbes (1970) using the same species. In addition, Dyeson (1970) describes presumptive TSH cells in the caudal half of the gland, particularly in the medio-ventral and ventro-lateral regions. The basophil-II described for Sceloporus is most likely the TSH cell on the basis of its staining reactions, distribution, relative lack of response to gonadectomy, estrogen injections and physiological changes concerned with ovarian maturation and ovulation.

Our inability to detect a second gonadotrope using the techniques described clearly does not eliminate the possibility of a second cell. The application of different techniques may well reveal its presence. However, the studies of Licht and Rosenberg (1969) and Licht (1970) suggest that there may be only one gonadotropic cell. Although two gonadotropic cells and two hormones are clearly involved in gonadal control in the higher vertebrates, the suggestion that there may be only one gonadotropic cell type in fish (Lagios, 1965; van Overbeeke and McBride, 1967; Leatherland, 1969; Mattheij, 1970) and anura (van Kemenade, 1969; van Oordt and de Kort, 1969) is gaining strong support. In addition, results of chemical fractionation of pituitary extracts suggest only one gonadotropic hormone in the fish Cyprinus carpio and the salmon (Fontaine and Gérade, 1963; Burzawa-Gérade and Fontaine, 1965, 1966; Yamazaki and Donaldson, 1968a and b; Burzawa-Gérarde, 1969).

TABLE 1

TINCTORIAL PROPERTIES OF VARIOUS CELL TYPES OF THE PARS DISTALIS OF *SCELOPORUS CYANOGENYS*

Staining Technique	Cell Type			
	A-I	A-II	B-I	B-II
Cleveland-Wolfe	bright orange-dull red	bright red-purple	pale purple-gray	bright blue
PAS-alcian blue (pH 0.8)-orange G	pale pink-orange	dull red	pink	pale purple
PAS-alcian blue (pH 3.0)-orange G	orange	red	magenta	blue-purple
Aldehyde-fuchsin with Halm's counterstain	orange	red-brown	pale tan-violet	violet

TABLE 2

HISTOLOGICAL CELL TYPES AND THEIR PROPOSED FUNCTIONS

Class	Distribution in anterior pituitary	Proposed Function
Acidophil-I	uniform	growth hormone (STH)
Acidophil-II	rostral	prolactin (LTH)
Basophil-I	uniform	gonadotropin (GTH)
Basophil-II	mid-region	thyrotropin (TSH)

PLATE 1

Figure 1. Pituitary gland of preovulatory Sceloporus cyanogenys. Cleveland-Wolfe trichrome. The position of the neural lobe (NL), intermediate lobe (IL), and anterior lobe (AL) is shown. Red-orange cells of the anterior lobe are acidophils type I (A-I) (general distribution). Red-purple cells are acidophils type II (A-II) (primarily rostral distribution). Orientation: D-dorsal, V-ventral, R-rostral, C-caudal. x250

Figure 2. Pituitary gland of preovulatory Sceloporus cyanogenys. PAS, alcian blue, pH 3.0, orange G. Dark blue cells are alcian positive basophils which are distributed throughout the gland. Purple cells in rostral region are acidophils type II which are PAS positive. x250

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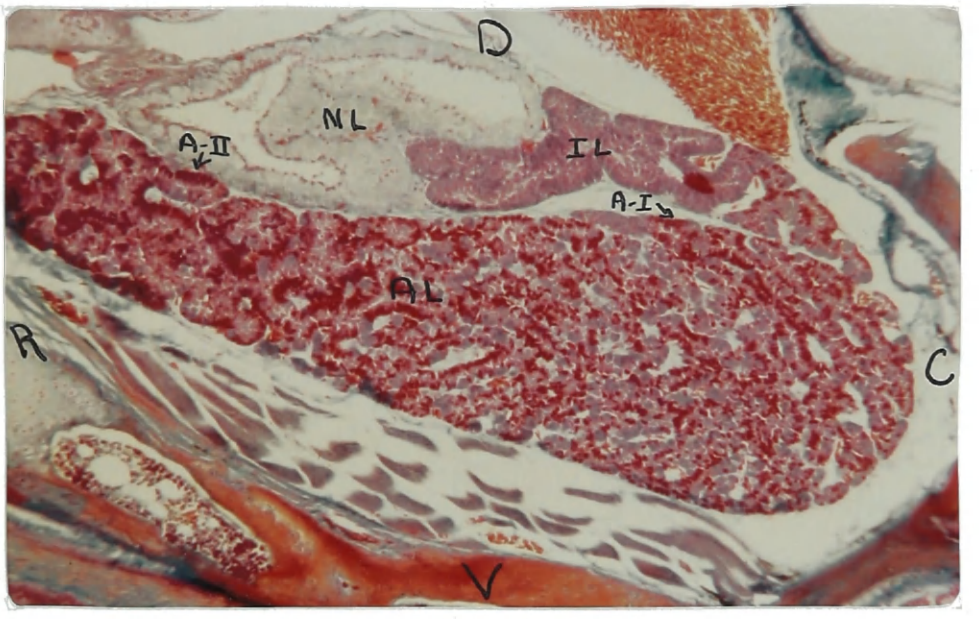
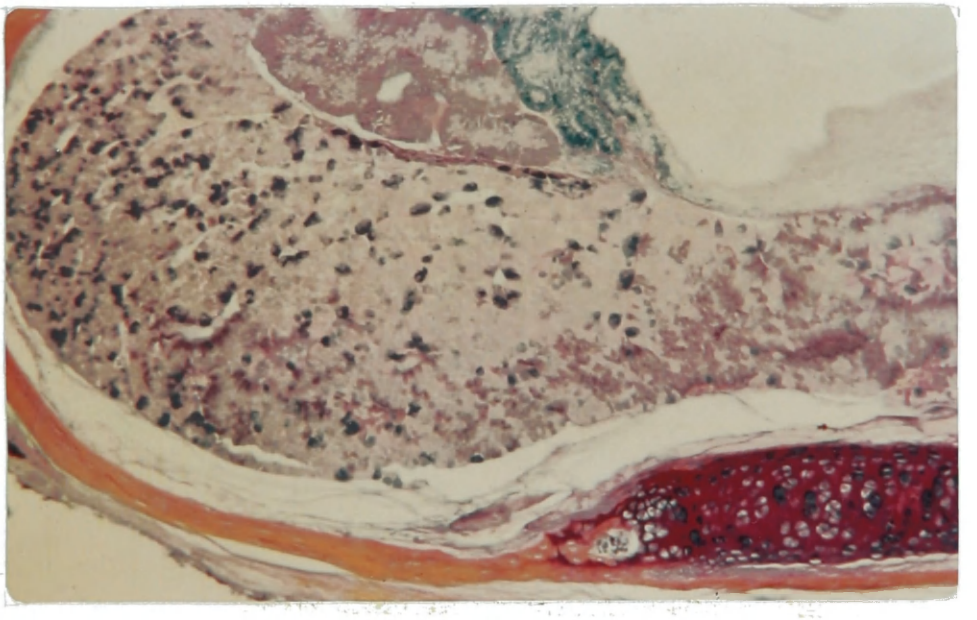




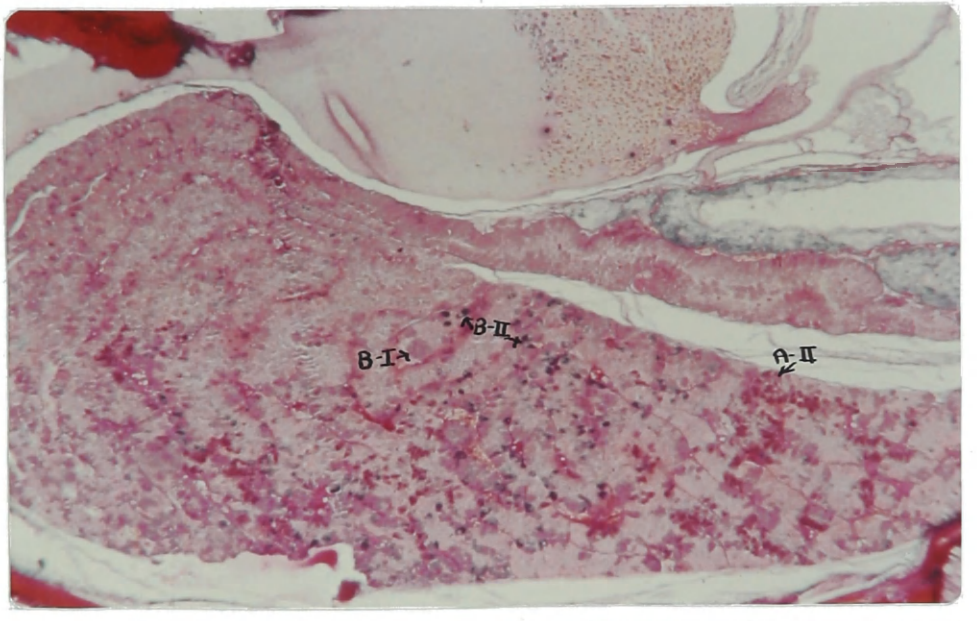
2

PLATE 2

Figure 3. Post-partum Sceloporus cyanogenys pituitary gland. PAS, alcian blue, pH 3.0, orange G. This shows the predominately mid-region and ventro-caudal distribution of basophil type II (B-II) cells which are dark blue. Intermixed with these are magenta cells (PAS positive) which are basophil type I (B-I) cells. These have a more general distribution. In the rostral region are red-purple cells, acidophil type II (A-II), which are PAS positive. x250

Figure 4. Pituitary is from the same animal as above but stained with Cleveland-Wolfe trichrome. All basophils stain with aniline blue, but there is a heavy concentration of granulation in cells of the mid-region where basophil type II cells are found. Acidophils type I (A-I) and type II (A-II) are also present. x250

3



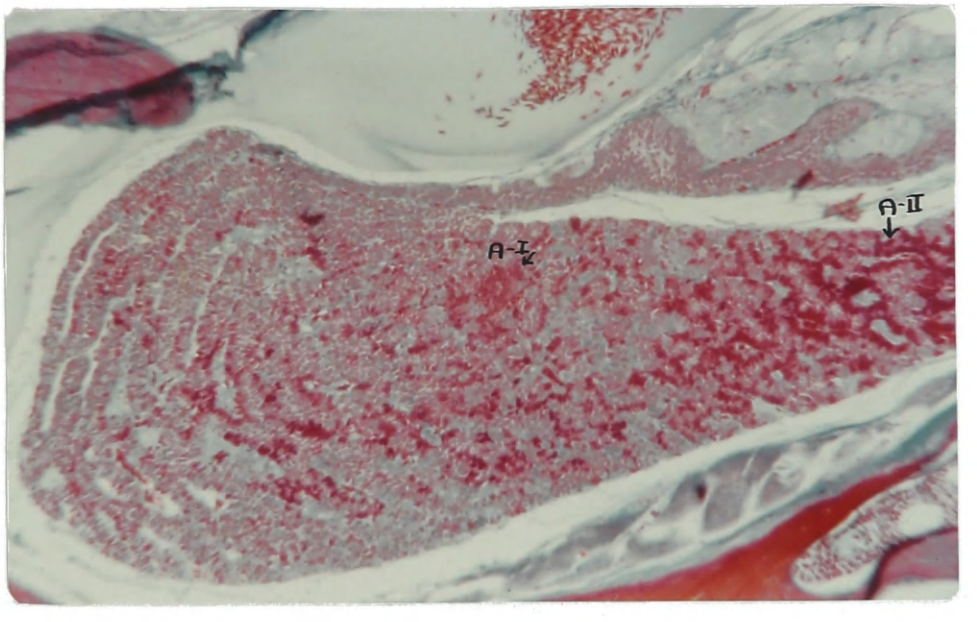


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PLATE 3

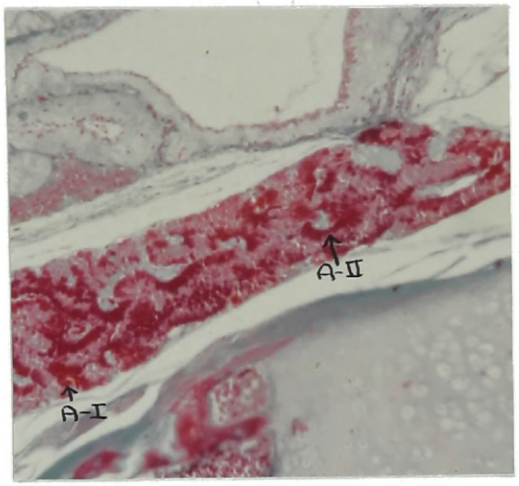
Figure 5. Pituitary of post-partum Sceloporus cyanogenys stained with Cleveland-Wolfe trichrome showing acidophil type II (A-II) cells which are red-purple. Acidophil type I (A-I) cells which are red-orange are intermixed with the type II cells. This is the rostral region of the pituitary shown in figure 4. x250

Figure 6. Post-partum pituitary of Sceloporus cyanogenys stained with Cleveland-Wolfe trichrome showing the larger rostral ($A\text{-}I_a$) and smaller caudal ($A\text{-}I_b$) subtypes of acidophil type I. x400

Figure 7. Post-partum pituitary of Sceloporus cyanogenys. Cleveland-Wolfe trichrome. Red-orange cells are acidophil type I (A-I) cells. Pale blue-grey cells are basophil type I (B-I). x1000

Figure 8. Post-partum pituitary of Sceloporus cyanogenys. PAS, alcian blue, pH 3.0, orange G. Light pink-orange cells are acidophil type I (A-I), red-purple cells are acidophil type II (A-II), magenta cells are basophil type I (B-I), and dark blue cells are basophil type II (B-II) cells. x1000

5



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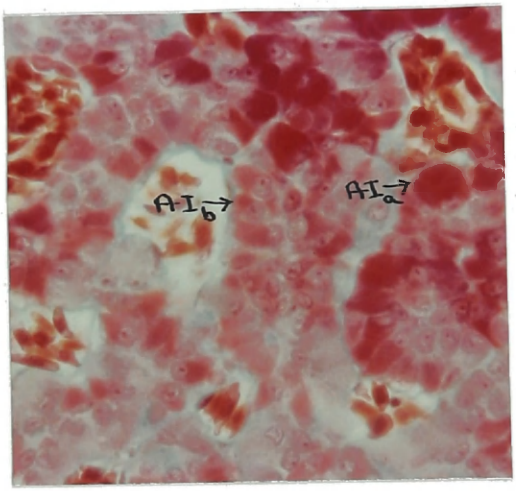

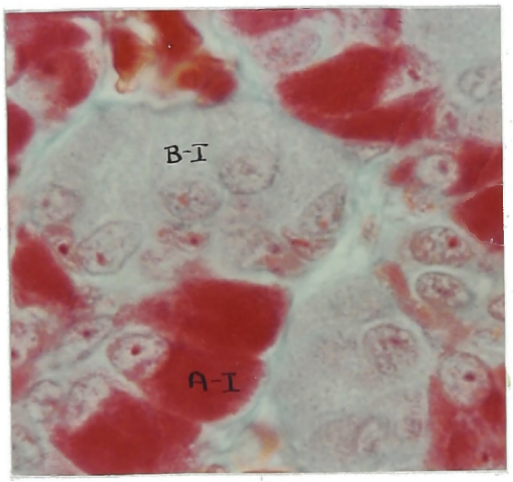

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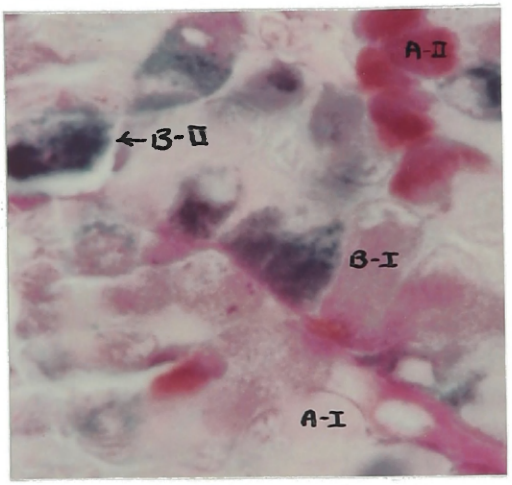

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PLATE 4

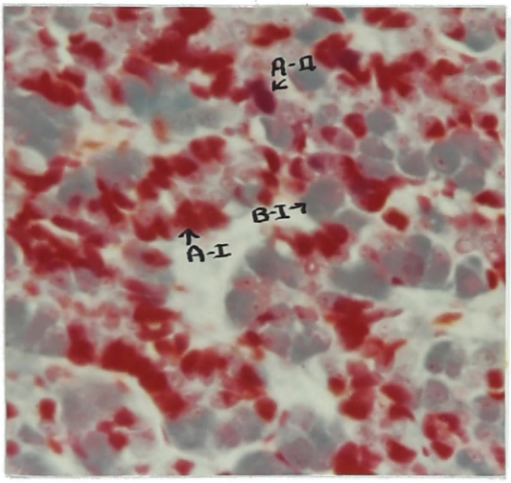
Figure 9. Preovulatory pituitary of Sceloporus cyanogenys. Cleveland-Wolfe trichrome. Basophils of type I (B-I) are numerous and well-granulated. Red-orange acidophils are type I (A-I). A few red-purple acidophils of type II (A-II) are present. x400

Figure 10. Pituitary of castrated female Sceloporus cyanogenys. Cleveland-Wolfe trichrome. Vacuoles in type I basophils (B-I) are numerous and in some signet ring cells (s) are present. Red-orange acidophil type I (A-I) cells are also present. In this animal they appear to be somewhat smaller and fewer in number. x400

Figure 11. Pituitary of castrated female Sceloporus cyanogenys. Cleveland-Wolfe trichrome. This shows signet ring cells (s) which are exhausted basophil type I cells. Red-orange cells are acidophil type I cells. x1000

Figure 12. Pituitary of castrated female Sceloporus cyanogenys which had been estrogen injected. Cleveland-Wolfe trichrome. Red-orange cells, acidophil type I (A-I), appear in extremely large numbers. Basophil type I (B-I) cells are vacuolated but not to the extent of those in castrated animals with no estrogen injection (figure 10). x400

9



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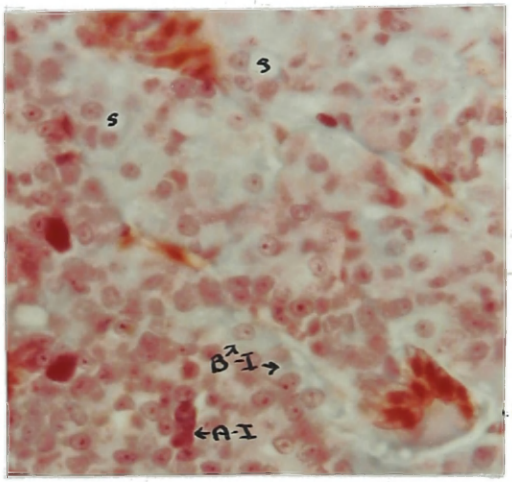

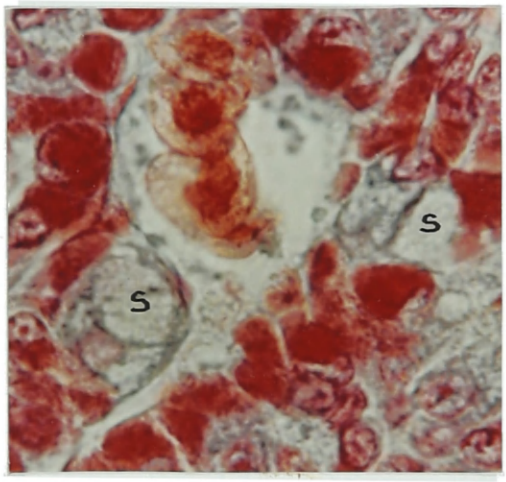

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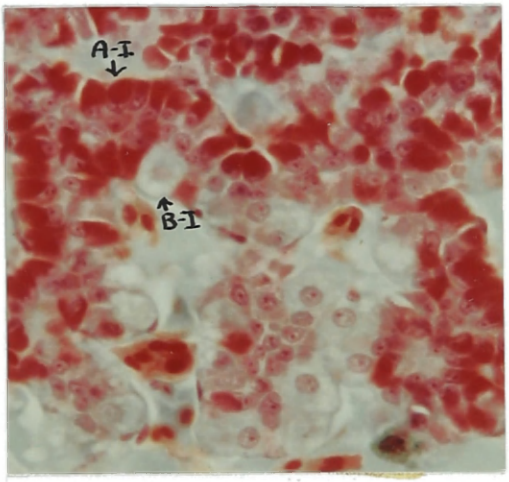

12

APPENDIX

Staining proceedures

Cleveland-Wolfe trichrome

reagents	time
1. xylene	5 minutes
2. xylene	5 minutes
3. 100% ethanol	5 minutes
4. 95% ethanol	2 minutes
5. 80% ethanol	2 minutes
6. 70% ethanol	2 minutes
7. Lugol's iodine solution (70% ethanol)	15 minutes
8. 5% sodium thiosulfate (aqueous)	2 minutes
9. distilled water	3 minutes
10. distilled water	3 minutes
11. distilled water	3 minutes
12. Erlich hematoxylin	5 minutes
13. distilled water	1 minute
14. acid alcohol (70% ethanol plus HCl)	until pink
15. alkaline alcohol (70% ethanol plus NH_4OH)	until blue
16. tap water	2 minutes
17. distilled water	2 minutes
18. 1% erythrosin (aqueous)	5 minutes
19. tap water	2 minutes
20. distilled water	2 minutes
21. 2% orange G in 1% phosphotungstic acid	20 seconds
22. tap water	rapid rinse
23. distilled water	rapid rinse
24. 0.05% aniline blue (aqueous) pH 3.0-4.0	2 minutes
25. 100% ethanol	rapid rinse
26. 100% ethanol	1 minute
27. xylene	3 minutes
28. xylene	5 minutes

PAS, alcian blue, orange G (alcian blue at pH 0.2 and pH 3.0)

reagents	time
1. xylene	5 minutes
2. xylene	5 minutes
3. 100% ethanol	5 minutes
4. 95% ethanol	2 minutes
5. 80% ethanol	2 minutes
6. 70% ethanol	2 minutes
7. Lugol's iodine solution (70% ethanol)	15 minutes
8. 5% sodium thiosulfate (aqueous)	2 minutes
9. distilled water	3 minutes
10. distilled water	3 minutes
11. distilled water	3 minutes
12. Gomori's mixture (see below)	3 minutes
13. 5% sodium bisulfite (aqueous)	until bleached
14. running tap water	1 minute
15. distilled water	1 minute
16. 1% alcian blue (see below)	30 minutes
17. running tap water	1 minute
18. 1% periodic acid	5 minutes
19. running tap water rinse	rinse
20. Schiff's reagent	15 minutes
21. running tap water	15 minutes
22. distilled water	1 minute
23. Erlich hematoxylin	5 minutes
24. distilled water	1 minute
25. acid ethanol (70% ethanol plus HCl)	until pink
26. alkaline ethanol (70% ethanol plus NH_4OH)	until blue
27. tap water	2 minutes
28. distilled water	2 minutes
29. 2% orange G in 1% phosphotungstic acid	20 seconds
30. tap water	rapid rinse
31. distilled water	rapid rinse
32. 90% ethanol	1 minute
33. 100% ethanol	2 minutes
34. 100% ethanol	3 minutes
35. xylene	3 minutes
36. xylene	5 minutes

Gomori's mixture
stock solutions: 2.5% potassium permanganate, 5% sulphuric acid
Mix 10 ml. of each of above stock solutions, add 60 ml. distilled water. Mixture keeps 2-3 days. It should turn sections chestnut brown.

PAS, alcian blue, orange G (continued)

Alcian blue
For pH approximately 3.0, dissolve 1 g. alcian blue in 100 ml. distilled water, add 1 ml. glacial acetic acid.
For pH approximately 0.2, dissolve 1 g. alcian blue in 100 ml. 10% sulphuric acid.

PAS, orange G

The staining proceedure is the same as for PAS, alcian blue, orange G (above) omitting steps 12 through 17.

Gabe's aldehyde-fuchsin with Halmi's counterstain

reagents	time
1. xylene	5 minutes
2. xylene	5 minutes
3. 100% ethanol	5 minutes
4. 95% ethanol	2 minutes
5. 80% ethanol	2 minutes
6. 70% ethanol	2 minutes
7. Lugol's iodine solution (70% ethanol)	15 minutes
8. 5% sodium thiosulfate (aqueous)	2 minutes
9. distilled water	3 minutes
10. distilled water	3 minutes
11. distilled water	3 minutes
12. Gomori's mixture (see PAS, alcian blue, orange G staining proceedure above)	1 minute
13. 2% sodium bisulfite (aqueous)	1 minute
14. running tap water	30 seconds
15. Gabe's AF (see below)	5 minutes
16. acid alcohol (70% ethanol plus HCl)	rinse
17. running tap water	1 minute
18. Erlich hematoxylin	5 minutes
19. acid alcohol (70% ethanol plus HCl)	until pink
20. alkaline alcohol (70% ethanol plus NH_4OH)	until blue
21. tap water	2 minutes
22. distilled water	2 minutes
23. Halmi's counterstain (see below)	15 seconds
24. 70% ethanol	rinse
25. 95% ethanol	rinse
26. 100% ethanol	1 minute
27. 100% ethanol	2 minutes
28. xylene	3 minutes
29. xylene	5 minutes

Gabe's aldehyde-fuchsin with Halmi's counterstain (continued)

Gabe's AF
Pour 200 ml. boiling water over 1 g. basic fuchsin. Boil ± 1 minute. (2) Cool and filter. (3) Add 2 ml. concentrated HCl and 2 ml. paraldehyde. Keep in well-stoppered bottle at room temperature. (4) Follow changes taking place in the fluid by daily pouring a drop on a piece of blotting paper. As soon as red color disappears solution is ripe. ± 4 days at 20°C. (5) Filter. (6) Dry the blotting paper plus precipitate from filtration (to remove HCl and paraldehyde). (7) Make a saturated solution in 70% alcohol (± 150 ml.). This is the stock solution. It keeps in daylight and room temperature for about one year. Before use 75 ml. 70% alcohol plus 1 ml. glacial acetic acid plus 25 ml. of the stock solution are mixed to make staining solution.

Halmi's counterstain
Dissolve 0.2 g. light green SF yellowish, 1.0 g. orange G, 0.5 g. phosphotungstic acid, 0.5 g. chromotope 2 R and 1 ml. glacial acetic acid in 100 ml. distilled water.

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